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RESEARCH LABORATORY
RESEARCH TRIANGLE PARK, NC 27711

OFFICE OF
RESEARCH AND DEVELOPMENT

MEMORANDUM

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Subject: Analysis of the Motor Activity Data from the Rat Developmental Neurotoxicology Study

From: Kevin M. Crofton, Ph.D.
Neurobehavioral Toxicology Branch

and

Robert C. MacPhail, Chief
Neurobehavioral Toxicology Branch, MD-74B
Neurotoxicology Division
National Health Effects and Environmental Research Laboratory

and

Hugh A. Tilson, Director
Neurotoxicology Division
National Health Effects and Environmental Research Laboratory

To: Annie Jarabek
National Center for Environmental Assessment

This memo comments on the motor activity data found in the developmental neurotoxicology study of ammonium perchlorate (Argus Protocol #1613-002; Study#7757A210-1096-25F). The main issue is one of data interpretation and the interplay between biological and statistical significance as it relates to the increased motor activity observed in the perchlorate exposed 14 day old male rats.

The data were originally analyzed by the contractor using two separate three-way ANOVAs (Age, Treatment, and Habituation-Block), one for each gender. This analysis demonstrated a significant decrease in the amount of habituation in the two highest dosage

groups. In the my original review I recommended that an additional analysis of the data be conducted using gender as a within-subjects variable, or alternatively, using a nested design with gender nested under litter (see Holson and Pearce, 1992 and Cox, 1994 for a review of statistical methods used in developmental studies and the importance of using litter as the unit of measure). This review also questioned why the method and/or statistics did not detect any significance to the dosage-dependent increase in total session counts that amounted to a 95% increase over controls in the highest dosage group (see Figure 1). The response from Argus (York, 1998; letter dated October 2, 1998) included a new analysis in which gender was used as a between subjects variable. No interactions with, or main effects of, treatment were found in this analysis. Please note that the subsequent secondary analysis submitted is still not correct. It was requested that gender be nested under litter, not used as a between subjects variable.

Regardless of the adequacy of the analyses, the contractor and sponsor failed to respond adequately to the request for an explanation of why the analysis failed to detect significance in the PND14 motor activity for the male rats (see Figure 1). This graphic illustrates the clear dosage-dependent increase in two different measurements of motor activity, time-spent-in-movement and total number-of-movements. The time variable increased over 95% at the highest dosage relative to controls (group means of 363 and 186, respectively). The number-of-movements variable increased approximately 65% relative to controls. In our opinion, and likely the opinion of most developmental neurotoxicologists, increases in motor activity over 50%, especially in developing animals, are clearly of concern from a biological perspective.

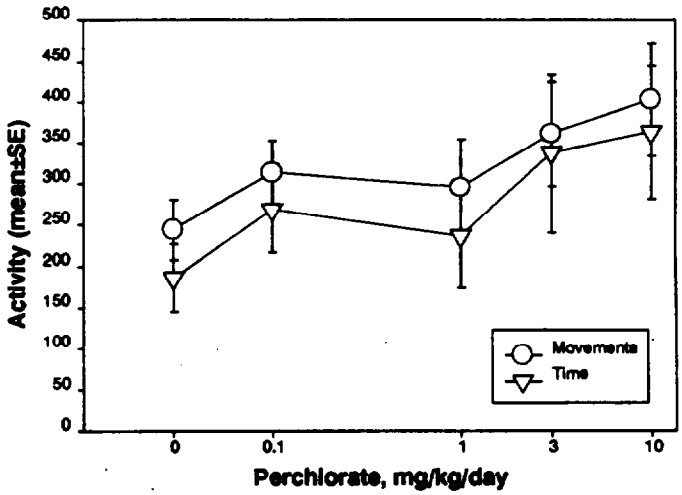

Figure 1. The effects of developmental exposure to perchlorate on motor activity in male rats on postnatal day 14. The dosage-dependent increases in both number of movements and time spent in movement were not statistically different even though the increases were substantial at the higher dosages.

The critical issue presented by these data for risk assessment is how to resolve the difference between what is clearly a biologically significant alteration in behavior with the lack of statistical significance. In regards to resolving this problem the Agency requested positive control data from the testing laboratory for this device (not available in the original report), as well as any available historical control data. The sponsor and the contractor replied with a number of positive control studies and a limited amount of historical control data for motor activity from PND14 rats.

The positive control data was requested to help understand the sensitivity of the device in detecting increases in motor activity (i.e., what is the smallest increase in motor activity that has been detected by this device). Unfortunately, the positive control data were of limited use in interpreting the sensitivity of the device. The submission (appendix to original report) contained data from experiments with amphetamine and triadimefon in adult rats. The smallest increase in activity that was induced by either chemical was a 109% increase relative to controls. While

these effects were statistically significant, they are greater than the effects produced by the highest dosage of perchlorate in the PND14 animals. There were also positive control data from chlorpromazine-treated animals that showed significant decreases in activity of 32% or more. However, ability to detect decreases does not necessarily translate to increases.

The historical-control data from PND14 rats was requested to help understand the variability normally found in control animals. Unfortunately, the historical-control data submitted were only useful in that the data raised more suspicion that the degree of experimental control over this behavior by the testing facility was inadequate. For the Time data the control mean for the perchlorate data set was 186. For the three relevant historical control data sets the means were 1026, 965 and 458. Either the lab has very little control over the behavior, or the data are from a different test apparatus or from a different usage of the same apparatus. In any case the data are of no use in helping to determine the historical profile of control animals behavior in this test apparatus.

In lieu of the lack of useful positive control and/or historical control data, we are still left with the issue of statistical versus biological significance. There are a number of reasons for the lack of statistical significance. The first reason is the extremely large within-group variability exemplified by coefficients of variation (CV) of greater than 100% . In our opinion this is likely due to the inability of the testing laboratory to gain adequate control over the behavior being tested. This large variability results in very little statistical power and increases the potential for Type II errors. Normally an increase in sample size (by additional testing) allows for adequate power to refute or support the conclusion of an effect. Given the CVs of about 100% , simple power calculations (see Cohen, 1987) for detecting a 40% change in one group out of five results in needed group sizes of about 70-90 animals per group. The second reason is that the effect, a 95% increase, while rather large from a biological perspective, occurs in only one gender on only one day out of 4 test days. The large variability coupled with the complicated design (Treatment, Age, Gender, Block) will tend to wash out anything other than extremely large effects. This conclusion is consistent with the content of a phone conversation (dated 11/04/98) with Dr. Simon Mats. Dr. Mats is the statistician from the contract lab (Pimedica/Argus) who conducted the revised statistical analysis of these data. Lastly, the effect seen in the males on PND14 may indeed be a Type I error and would not be found again if this experiment was repeated.

The conclusion of a ***biological significance*** to the effect seen in this report is supported by both the underlying mechanism-of-action of perchlorate and the effects of other chemical and physical insults on the motor activity of postnatal rats. The hypothesis that a thyrotoxic chemical would induce a delay in any aspect of nervous system development is highly plausible. Developmental exposure to numerous hypothyroid inducing agents (e.g., propylthiouracil, methimazole) are known to result in delays in the ontogeny in many behaviors (cf., Comer and Norton, 1982; Goldey et al., 1995; Schneider and Golden, 1987; Tamasy et al., 1986) including the development of habituation. However, effects of these chemicals on total motor activity counts vary from increased to decreased, depending on the chemical and age of testing. The mechanism for the gender-dependent nature of the effect of perchlorate also remains to be determined. In addition, there are numeous reports from the literature that support the biological significance of a 40-50% increase in motor activity in postnatal rats (cf., Campbell et al., 1969; Ruppert et al., 1984; 1985).

In summary, it is our opinion that the increase in activity should be considered

biologically significant until additional data can be marshaled to suggest/prove otherwise. The inadequacy of standard parametric statistics to detect a significant difference suggests that alternative analyses should be used on these data, such as the benchmark approach. This type of statistical approach may be useful because of the inverse relationship between the data variability and the BMD.

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